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Island hopping, long-distance dispersal and species radiation in the Western Indian Ocean: historical biogeography of the Coffeae alliance (Rubiaceae)

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30 **ABSTRACT**

31

32 **Aim** The Western Indian Ocean region (WIOR), is home to a very diverse and largely unique
33 flora that has mainly originated via long-distance dispersals. The aim of this study is to gain
34 insight into the origins of the WIOR biodiversity and to understand the dynamics of
35 colonization events between the islands. We investigate spatial and temporal hypotheses of
36 the routes of dispersal, and compare the dispersal patterns of plants of the Coffeae alliance
37 (Rubiaceae) and their dispersers. Rubiaceae is the second most species-rich plant family in
38 Madagascar, and includes many endemic genera. The neighbouring archipelagos of the
39 Comoros, Mascarenes and Seychelles also harbour several endemic Rubiaceae.

40

41 **Location** The islands of the Western Indian Ocean.

42

43 **Methods** Phylogenetic relationships and divergence times were reconstructed from plastid
44 DNA data of an ingroup sample of 340 species, using Bayesian inference. Ancestral areas and
45 range evolution history were inferred by a maximum likelihood method that takes topological
46 uncertainty into account.

47

48 **Results** At least 15 arrivals to Madagascar were inferred, the majority of which have taken
49 place within the last 10 Myr. Most dispersal events were supported as being from mainland
50 Africa, but *Catunaregam* may have dispersed from Asia. Although most Coffeae alliance
51 lineages are zoochorous, the general pattern of dispersals from Africa is incongruent with the
52 biogeographic origins of the extant Malagasy volant frugivores. Several out-of-Madagascar
53 dispersals were inferred to the neighbouring islands, as well as back-colonizations of Africa.

54

55 **Main conclusions** The African flora has been of foremost importance as source of dispersal
56 to the islands of the Western Indian Ocean. Following the colonization of Madagascar, rapid
57 radiations appear to have taken place in some clades, and Madagascar has also been an
58 important source area for subsequent dispersal to the Comoros, Mascarenes and Seychelles.

59

60 **Keywords**

61 **angiosperm, Comoros, dispersal–extinction–cladogenesis, divergence times, island**

62 **biogeography, long-distance dispersal, Madagascar, Mascarenes, molecular dating,**
63 **Seychelles**

64

65

66 INTRODUCTION

67

68 The Western Indian Ocean region (WIOR) comprises Madagascar, the Comoros, Mascarenes
69 and Seychelles and has been recognized as a hotspot of global biodiversity (Myers *et al.*,
70 2000). As such, it has become a hotspot for biogeographical studies detailing the origins of
71 this diversity (e.g., Yoder & Nowak, 2006; Agnarsson & Kuntner, 2012; Samonds *et al.*,
72 2013). Madagascar in particular has long been considered to have an extraordinary
73 biodiversity – while some otherwise widely distributed taxa are notably absent, other groups
74 are very speciose (Simpson, 1940). Overall, the flora of Madagascar comprises more than
75 11000 species of vascular plants, 82% of which are endemic (Callmänder *et al.*, 2011).

76 The WIOR harbours both old continental islands and young volcanic islands with a
77 wide variety of habitats, offering an ideal system for studies of biodiversity formation (Losos
78 & Ricklefs, 2009; Vences *et al.*, 2009). The continental islands were formed following the
79 break-up of East Gondwana: Madagascar was isolated from the Indian subcontinent about 88
80 Ma, and drifted towards the equator alongside Africa (Storey, 1995; Storey *et al.*, 1995; Wells,
81 2003), and the granitic Seychelles were subsequently separated from India about 64 Ma
82 (Plummer & Belle, 1995; Collier *et al.*, 2008). Volcanic activity in the region gave rise to the
83 Mascarene islands, comprising Mauritius, Réunion and Rodrigues (8, 2 and 1.5 Ma
84 respectively; McDougall *et al.*, 1965; McDougall, 1971; Duncan, 1990). Older islands may
85 have existed in the trail of the Réunion hotspot in the past, but have since been eroded and
86 submerged (Gardner, 1986; Warren *et al.*, 2010). The Comoros archipelago is also the result
87 of recent volcanism, of which the oldest island is Mayotte, about 8 Ma (Nougier *et al.*, 1986).
88 In addition to the continental and volcanic islands there are also small coralline islands, such
89 as the Aldabra atoll (Seychelles).

90 Although it has long been recognised that the flora of Madagascar is affiliated with the
91 African flora, biogeographic theories on the origin of the island's biodiversity have
92 emphasized its Gondwanan heritage, as "a center of survival for archaic autochthonous
93 plants" (Koechlin, 1972; Leroy, 1978; p.583). The historical proximity to the Indian

94 subcontinent as well as extant phylogenetic connections between Madagascar and Asia have
95 also prompted theories of biotic exchange between these regions by an isthmian land
96 connection ("Lemurian land bridge"; Van Steenis; 1962; p. 343; Rage, 2003), or by island
97 stepping-stones (Schatz, 1996; Warren *et al.*, 2010). However, recent studies have highlighted
98 the importance of long-distance dispersal, and indicated that the majority of the extant biota in
99 Madagascar descended from African ancestors that arrived during the Cenozoic (e.g., Yoder &
100 Nowak, 2006; Buerki *et al.*, 2013; Samonds *et al.*, 2013). Madagascar has in turn been an
101 important source of dispersal to the neighbouring archipelagos of the Comoros, Mascarenes
102 and Seychelles (e.g., Micheneau *et al.*, 2008; Le Péchon *et al.*, 2010; Wikström *et al.*, 2010).
103 Despite much recent interest in the biogeography of Madagascar, there are few in-depth
104 studies of the spatio-temporal history of its flora (Vences *et al.*, 2009).

105 Next to Orchidaceae, Rubiaceae is the most species-rich family in Madagascar, also in
106 terms of number of endemic species (Callmander *et al.*, 2011). In this study, we focus on the
107 Coffeae alliance of subfamily Ixoroideae (Razafimandimbison *et al.*, 2011; Kainulainen *et*
108 *al.*, 2013). In the WIOR, this clade is represented by the tribes Alberteae, Bertiereae,
109 Coffeae, Gardenieae, Octotropideae and Pavetteae. Many genera are endemic to Madagascar,
110 i.e., *Canephora*, *Chapelieria*, *Flagenium*, *Jovetia*, *Homollea*, *Lemyrea*, *Mantalania*,
111 *Melanoxerus*, *Nematostylis*, *Pseudomantalania*, *Razafimandimbisonia*, *Robbrechtia* and
112 *Schizenterospermum*, but endemic genera are also found in the Mascarenes (*Fernelia* and
113 *Ramosmania*) and the Seychelles (*Paragenipa*). Other genera are widespread in the region –
114 *Coffea* and *Coptosperma*, for example, occur in all the archipelagos except the Seychelles,
115 and *Paracephaelis* and *Tarenna* are only absent from the Mascarenes.

116 Dispersal by birds is a commonly invoked explanation for the colonization of
117 Madagascar by fleshy-fruited plants (e.g., Renner, 2004), and the fruits of the Coffeae
118 alliance are with few exceptions fleshy. However, according to Hawkins & Goodman (2003;
119 and references therein), few species of birds in Madagascar are frugivores, and none of them
120 is migrational. Only seven species of forest-dwelling birds are listed as essential frugivores: a
121 bulbul and two species each of parrot, pigeon and asitie. Notably, it has been suggested that
122 these birds have an Asian or Australasian origin (Shapiro *et al.*, 2002; Warren *et al.*, 2005;
123 Moyle *et al.*, 2006; Schweizer *et al.*, 2010; although the ancestral area of the Madagascar
124 green pigeon is unknown).

125 Here we use an extensive sample of species from the WIOR and sequence data of

126 multiple molecular markers in order to reconstruct the biogeographic histories of the tribes of
127 the Coffeae alliance in the WIOR and understand how, when and from where they have
128 colonized this region. Specifically, we investigate (1) predominance of African or Asian
129 dispersal events to the WIOR; (2) incidence and direction of stepping-stone dispersal; (3)
130 evidence of back-colonization from islands to continents; and (4) if the biogeographic patterns
131 of plants and their presumed seed dispersers are congruent.

132

133

134 MATERIALS AND METHODS

135

136 Taxon sampling

137

138 Efforts were made to sample all 27 species of the Coffeae alliance present on the Comoros,
139 Mascarenes and Seychelles, and at least one representative of the 26 genera known from
140 Madagascar. However, attempts to sequence DNA from the Malagasy endemic
141 *Pseudomantania* were unsuccessful. Also missing are *Polysphaeria lanceolata* subsp.
142 *comorensis* from Anjouan, *P. multiflora* from Aldabra and *Coptosperma mitochondrioides*
143 from Mayotte. Sampling density was increased in species-rich and widespread genera such as
144 *Coffea*, *Gardenia*, *Rothmannia* and *Tarenna*. Of the 55 genera of the Coffeae alliance
145 occurring in Africa, 49 were sampled, as were 32 of the 52 genera found in Asia, Australasia
146 or the Pacific. In contrast, the sampling of Neotropical taxa was limited (*Bertiera* is the only
147 genus of the Coffeae alliance present both in the Indian Ocean and the Neotropics). The
148 Malagasy sampling included nine as of yet undescribed species of *Hyperacanthus*, and at least
149 10 undescribed taxa of Pavetteae. The sole species of the Coffeae alliance found on Socotra
150 (*Kraussia socotrana*) was also included. All 12 extant species from the Mascarenes were
151 sampled along with nine out of 11 species from Comoros and four out of five species from the
152 Seychelles. Eighty-five out of 159 currently recognized Malagasy species were included
153 (although the estimated number of species on Madagascar is at least 240). In total, the taxon
154 sampling comprised 387 specimens from 364 species. An overview of species names, voucher
155 information and GenBank accession numbers is given in Appendix S1 in the Supporting
156 Information. The nomenclature follows that of Govaerts *et al.* (2013), with a few exceptions
157 (i.e., *Empogona ruandensis*, *Pelagodendron vitiense*, *Sukunia pentagonioides* and *Tarenna*

158 *malacophylla*), and the tribal classification follows that of Mouly *et al.* (2014).

159

160

161 **DNA extraction, amplification and sequencing**

162

163 DNA was extracted following the protocol of Doyle & Dickson (1987), and purified using the
164 QIAquick® PCR purification kit (Qiagen, Hilden, Germany). Four plastid DNA regions were
165 chosen for this study: the *rps16* intron, *ndhF-trnL* (UAG), *trnK* intron (including *matK*) and
166 *trnT-trnF*; all amplified using the primers and PCR protocol detailed by Kainulainen &
167 Bremer (2014). PCR products were cleaned using Multiscreen Filter plates (Millipore,
168 Billerica, Massachusetts, U.S.A.), sequenced using the amplification primers and the
169 BigDye® terminator cycle sequencing kit, and subsequently analysed on a ABI PRISM 3100
170 Genetic Analyzer (Applied Biosystems, Foster City, California, U.S.A.). Alternatively,
171 sequencing was done using the EZ-seq v.2.0 sequencing service provided by Macrogen
172 Europe (Amsterdam, The Netherlands). Sequence reads were assembled using the STADEN
173 PACKAGE v1.5.3 (Staden, 1996; Staden *et al.* 2000), and sequences new to this study (1172)
174 were deposited in GenBank (Appendix S1 in the Supporting Information). Additional
175 sequences (286) were obtained from GenBank (for references, see Appendix S1). *AccD*, *petD*
176 and *rpl16* data for Bertiereae and Coffeae (166 sequences in total; Appendix S1) were also
177 added to the data set from Davis *et al.* (2007, 2011), Maurin *et al.* (2007) and Tosh *et al.*
178 (2009).

179

180

181 **Phylogenetic analyses**

182

183 Sequence data were aligned using MUSCLE 3.8.31 (default settings; Edgar, 2004), after having
184 been sorted by size using BIOEDIT (Hall, 1999). The latter program was also used to edit the
185 alignments; alternative sequence versions of suspected sequence inversions were separated
186 from each other in the alignments (but not excluded from the analyses; i.e., corresponding to
187 positions 114471–114508 (KJ816005–6, KJ816018), 114501–114524 (KJ816122), 114899–
188 114990 (KJ136920), 114585–114592 (KJ816044, KJ816046–7) and 115063–115083
189 (KJ136903) of the *Coffea arabica* L. plastid genome (GenBank accession number, EF044213;

190 Samson *et al.*, 2007; all within the *rpl32* region). Homoplastic sequence inversions in *matK*,
191 *rps16*, *accD* and *petD*, corresponding to positions 2022–2023, 5412–5430, 60199–60216 and
192 77852–77866 of EF044213, respectively, were treated in the same manner.

193 Phylogenetic reconstructions were done using Markov chain Monte Carlo (MCMC)
194 methods (Yang & Rannala, 1997). Bayesian analyses were conducted using MRBAYES 3.2.2
195 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) and BEAST 1.8 (Drummond
196 & Rambaut, 2007; Drummond *et al.*, 2012). Data were treated as a single partition, and the
197 GTR + I + G nucleotide substitution model was chosen based on the corrected Akaike
198 information criterion (AICc) as calculated using the program jMODELTEST 2.1.6 (Darriba *et*
199 *al.* 2012). The MRBAYES analysis comprised four runs of four chains each that were run for 5
200 $\times 10^6$ generations, the first 25% of which were excluded as burn-in.

201 Divergence times were estimated using BEAST 1.8, implementing the uncorrelated
202 lognormal clock model and the birth-death tree prior (Gernhard, 2008). The root node was
203 assigned a normally distributed age prior with a mean of 96 Ma and a standard deviation of
204 4.6 Myr; secondary calibration priors were similarly applied to the nodes representing the
205 most recent common ancestor (mrca) of Rubiaceae (87 ± 4.6 Ma), Ixoroideae (59 ± 7.2 Ma)
206 and the core Ixoroideae (36 ± 4.6 Ma); all strongly supported nodes. These age priors conform
207 to the 95% highest posterior density (HPD) interval of corresponding nodes inferred in a
208 study of Rubiaceae divergence times by Wikström *et al.* (2015). The BEAST analysis
209 comprised eight runs of 50×10^6 generations. The individuals runs were combined and
210 resampled (every 20000th generation) after removal of the burn-in (25%) to produce a sample
211 of 15000 trees. Effective samples sizes of the parameters were evaluated using the program
212 TRACER 1.6 (Rambaut & Drummond, 2013). Mean node ages and age density intervals were
213 summarized on the sampled topology of the maximum product of clade credibilities. All
214 analyses were performed on the Cipres science gateway portal v3.3 (Miller *et al.*, 2010).

215

216

217 **Biogeographic analyses**

218

219 Seven geographic areas were recognized in the biogeographic analyses: mainland Africa (A),
220 Americas (B), Asia (C; including Australasia and the Pacific), Comoros (D), Madagascar (E),
221 Mascarenes (F) and the Seychelles (G). Distribution data were obtained from the World

222 checklist of Rubiaceae (Govaerts *et al.*, 2013). Undescribed or undetermined taxa were coded
223 according to where they were collected, as were samples of species occurring in more than
224 one geographic area (i.e., typically the species of interest and therefore more extensively
225 sampled across their distribution). The geographic range evolution of the group was
226 reconstructed using the dispersal–extinction–cladogenesis (DEC) model of Ree & Smith
227 (2008) implemented in the program LAGRANGE 20130526. The DEC model was
228 unconstrained (transitions between all areas equally likely) with a maximum of two areas per
229 node. In order to account for topological uncertainty, we compiled the likelihood
230 reconstructions for each node across a subsample of 100 trees randomly selected from the
231 posterior distribution of BEAST trees.

232

233

234 RESULTS

235

236 Clade posterior probabilities inferred by the two Bayesian MCMC methods (MRBAYES and
237 BEAST) did not differ greatly; both are reported in Appendix S2 in the Supporting Information,
238 along with mean divergence times, 95% HPD intervals and the results of the biogeographic
239 analyses. The maximum clade credibility (MCC) tree from the BEAST analyses is shown in
240 Figures 1–5. The inferred phylogenetic hypothesis was overall congruent with that of previous
241 molecular studies (e.g., Kainulainen *et al.*, 2013; Mouly *et al.*, 2014). The results of the
242 biogeographic analyses using the DEC model are summarized in Appendix S2 in the
243 Supporting Information.

244 Asia was inferred as the most likely ancestral area for the Coffeae alliance clade, but
245 Africa was supported as the ancestral area for the tribes Alberteae, Bertiereae, Coffeae,
246 Gardenieae, Octotropideae and Pavetteae, that are present in the WIOR. Multiple independent
247 dispersal events were found between mainland Africa and Asia, represented by the
248 *Discospermum-Xantonnea* clade, *Diplospora* and a clade in *Coffea* (Coffeae, Fig. 2); the
249 *Hyptianthera-Morindopsis* clade (Octotropideae, Fig. 3); *Benkara*, the *Brachytome-*
250 *Catunaregam* clade and clades in both *Aidia* and *Rothmannia* (Gardenieae, Fig. 4); and clades
251 in both *Pavetta* and *Tarenna* (Pavetteae, Fig. 5). Dispersal events from mainland Africa to the
252 Americas were also inferred, represented by Cordiereae, a clade in *Bertiera* and the *Randia-*
253 *Tocoyena* clade (Gardenieae). Early range evolution within the *Genipa-Gardenia* clade could

254 not be unequivocally resolved.

255 In the WIOR, the ancestral area reconstructions indicated at least 15 dispersals to
256 Madagascar, 8–11 dispersals out of Madagascar, four dispersals to the Mascarenes, four
257 dispersals to the Seychelles and 8–9 dispersals to the Comoros. Most dispersals have occurred
258 within the last 10 Myr, but at least the colonization of Madagascar by the
259 *Razafimandimbisonia-Nematostylis* clade is older (crown age 21.6 Ma; HPD, 13.7–29.1 Ma).
260 Support for a monophyletic Alberteae was low (Fig. 1), but the biogeographic analyses
261 favoured a single dispersal event from Africa to Madagascar. Africa was also indicated as the
262 ancestral area of the Indian Ocean radiation of *Bertiera* (Fig. 2). A single dispersal event into
263 the WIOR was supported, but whether Madagascar or the Mascarenes were colonized first
264 was equivocal.

265 At least four dispersals to Madagascar were inferred in Coffeae. However, patterns of
266 range evolution in *Coffea* were inconclusive, mainly due to topological uncertainty. The
267 species from Madagascar were not supported as monophyletic; the majority of the sampled
268 species formed a clade together with mainly East African species, whereas a *Coffea grevei*-*C.*
269 *humbertii* clade was resolved as more early divergent. The biogeographical interpretation
270 largely depended on the relationships among these clades and that of a clade comprising the
271 Mascarene species (*Coffea macrocarpa*, *C. mauritiana* and *C. myrtifolia*) together with *C.*
272 *mongensis*. The DEC analyses based on the MCC topology favoured a vicariance scenario in
273 which the ancestor of the East African-Malagasy clade was distributed in both these areas
274 (A|AE: 0.97). The Mascarene *Coffea* clade was supported as being the result of an
275 independent dispersal event from Africa.

276 *Empogona ovalifolia* dispersed from Africa to the Comoros and to Madagascar. The
277 biogeographic analyses favoured two independent dispersal events, although support within
278 the *Empogona ovalifolia* clade was low. Phylogenetic support within *Tricalysia* was poor and
279 consequently the biogeographic results were uncertain for this clade. At least two independent
280 arrivals to Madagascar were indicated: (1) *T. madagascariensis* and (2) a weakly supported
281 clade comprising the remaining sampled Malagasy species as well as the two African species
282 *T. capensis* and *T. jasminiflora*. The former represent a well-supported out-of-Africa dispersal
283 event, but biogeographic reconstructions of the latter clade were inconclusive because of
284 phylogenetic uncertainty.

285 At least four dispersals to Madagascar were reconstructed in Octotropideae (Fig. 3).

286 An out-of-Africa dispersal event was supported for *Mantalania*. The Malagasy Octotropideae
287 *Canephora*, *Chapeliera*, *Flagenium*, *Gallienia*, *Jovetia* and *Lemyrea* formed a clade together
288 with *Paragenipa* from the Seychelles. The ancestral area of this clade is Africa. Dispersal
289 from Madagascar to the Seychelles was inferred for *Paragenipa*. The genus *Galiniera* was not
290 resolved as monophyletic. The Malagasy *Galiniera myrtoides* formed a clade with East
291 African *Lamprothamnus zanguebaricus*, whereas *G. saxifraga* from Tropical Africa is grouped
292 with the South African *Burchellia bubalina*. Dispersal from Africa to Madagascar was
293 supported for the ancestor of *Galiniera myrtoides*. The Malagasy species of *Polysphaeria*
294 were not resolved as monophyletic. However, clade support within the genus was low. The
295 ancestral area of *Polysphaeria* was inferred as Africa. The DEC model favoured a vicariance
296 scenario for the MCC topology. Out-of-Africa dispersal was supported for the ancestor of the
297 Comoran *Polysphaeria multiflora*, as well as for *Cremaspora triflora* subsp. *comorensis*.

298 *Fernelia* and *Ramosmania* from the Mascarenes formed a well-supported clade nested within
299 a clade of African taxa, and Africa was the ancestral area of the *Fernelia-Ramosmania* clade.

300 Gardenieae included at least four dispersals to Madagascar (Fig. 4). The Malagasy
301 *Gardenia* species are monophyletic, nested within a clade of African species. Dispersal from
302 Africa to Madagascar was also inferred for *Melanoxerus*. In contrast, *Catunaregam* is nested
303 within a clade of Asian taxa. The genus is not supported as monophyletic, because the Indian
304 *Deccania pubescens* is nested within the clade as a poorly supported sister group to
305 *Catunaregam* sp. 2 from Madagascar. Out-of-Asia dispersal was supported for the latter
306 species. Phylogenetic relationships within *Hyperacanthus* were poorly supported, although a
307 clade comprising all sampled Malagasy species but one (*Hyperacanthus* sp. 7) was resolved
308 with strong support. The biogeographic analyses supported an African origin of the genus.
309 The DEC model favoured a vicariance scenario for the MCC topology. A single long-distance
310 dispersal from Africa to the Seychelles was inferred for *Rothmannia annae*.

311 The Indian Ocean Pavetteae are strongly supported as being monophyletic (Fig. 5),
312 and are nested within an African clade. The biogeographic analyses supported a single
313 dispersal to Madagascar, followed by many out-of-Madagascar dispersal events, including:
314 (1) dispersal to the Mascarenes in the *Coptosperma borbonicum-cymosum* clade; dispersals to
315 the Comoros in (2) *C. nigrescens*, (3) *C. supra-axillare*, (4) *Paracephaelis cinerea*, (5)
316 *Tarenna grevei* and (6) *T. spiranthera*; dispersals to the Seychelles in (7) *P. trichanta* and (8)
317 *T. sechellensis*; and dispersal back to Africa in the *C. littorale-rhodesiacum* clade.

318 *Coptosperma nigrescens* and *C. supra-axillare* have also dispersed to Africa, either via the
319 Comoros or independently from Madagascar.

320

321

322 DISCUSSION

323

324 The Lemurian land-bridge or stepping-stone hypothesis of dispersal postulates that biotic
325 exchange has occurred from Asia to Madagascar across the Indian Ocean by island hopping,
326 and via the Seychelles in particular (van Steenis, 1962; Schatz, 1996; Warren *et al.*, 2010).
327 Quaternary scenarios of the Indian Ocean geography at lower sea-levels estimated by Warren
328 *et al.* (2010), indicated that several submerged banks as well as the area that is now the
329 Seychelles archipelago, were large islands and subaerial during long time intervals (see also
330 Weigelt *et al.*, 2016). These islands should be considered in the study of the biogeographic
331 history of the Indian Ocean and may at least for birds have facilitated dispersal from Asia and
332 subsequent colonization of Madagascar (cf. Samonds *et al.*, 2013). However, our analyses
333 indicate that the "Lemurian" route of dispersal has been of less importance to the Coffeae
334 alliance, and that the predominant pattern of plant colonization in the WIOR is from mainland
335 Africa.

336 Although no stepping-stone dispersal was inferred via the Seychelles to Madagascar or
337 the rest of the WIOR, this study shows that, conversely, Madagascar has been important as a
338 stepping-stone for dispersal to the Seychelles (*Paracephaelis*, *Paragenipa* and *Tarenna*), as
339 well as to the Comoros (*Coffea*, *Coptosperma*, *Paracephaelis* and *Tarenna*) and the
340 Mascarenes (*Coptosperma* and probably *Bertiera*). That the Malagasy flora has been an
341 important source for colonizing the surrounding archipelagos has previously been shown in
342 several groups (e.g., Micheneau *et al.*, 2008; Le Péchon *et al.*, 2010; Strijk *et al.*, 2012; Stride
343 *et al.*, 2014). We also find evidence for back-colonization from Madagascar to mainland
344 Africa, a biogeographical pattern previously reported in plant families such as Gentianaceae
345 (Yuan *et al.*, 2005) and Celastraceae (Bacon *et al.*, 2016). Specifically, at least three
346 independent dispersal events to eastern Africa was inferred in *Coptosperma* (possibly also in
347 *Coffea* and *Tricalysia*).

348 Except Alberteae, which have wind-dispersed pterocarps, the fruits of the Coffeae
349 alliance are typically fleshy, and presumably zoochorous. In contrast to the Asian or

350 Australasian origins of the majority of Cenozoic bird introductions to Madagascar (Samonds
351 *et al.*, 2013, Table S1, and references therein), we find that Africa has been the foremost
352 source of plants of the Coffeae alliance in the region, a pattern that has been suggested as
353 being the predominant of most Malagasy plant groups (Yoder & Nowak, 2006). The
354 discrepant patterns in the origins of the extant frugivore birds and plants could indicate that
355 the African frugivorous birds have not been very successful in colonizing Madagascar, and
356 that the colonization opportunities for the African zoochorous plants may have been very
357 sporadic. Alternatively, the frugivorous bird species in question may have gone extinct. The
358 dispersal of seeds by fruit bats should also be considered in relation to long-distance dispersal
359 of fleshy fruits (e.g., Shilton *et al.*, 1999). However, as with the Malagasy frugivorous birds,
360 the fruit bat fauna is not very diverse: "rather limited in comparison with other Old World
361 tropical regions" (Hutcheon, 2003; p. 1205; in reference to the three species present in
362 Madagascar), and at least in the case of the flying foxes (*Pteropus*), they are recent arrivals
363 from Asia (O'Brien *et al.*, 2009). It is possible that rafting by washed-off mats of vegetation
364 has been the dominant means of seed dispersal in the region as has been suggested for non-
365 volant animals (Simpson, 1940; Ali & Huber, 2010).

366 All dispersals to Madagascar within the Coffeae alliance are from Africa, with the
367 exception of *Catunaregam* (Asia; Fig. 4) and possibly *Bertiera* (Mascarenes?; Fig. 2). The
368 first to disperse to Madagascar was likely the African ancestor of the *Nematostylis*-
369 *Razafimandimbisonia* clade (crown age 21.6 Ma; HPD, 13.7–29.1 Ma), followed by the
370 *Gallienia-Lemyrea* clade (crown age 10.4 Ma; HPD, 7.3–13.7 Ma) and the WIOR Pavetteae
371 (crown age 8.5 Ma; HPD, 6.5–10.7 Ma). The *Melanoxerus* clade has been present
372 Madagascar since 4.2 Ma (HPD, 1.9–6.9 Ma). The estimated crown age of *Mantalania* is only
373 3.0 Ma (HPD, 0.9–5.8 Ma), but the minimum age of colonization by this lineage would likely
374 have been older had *Pseudomantalania* also been included in the analyses. It is not clear if
375 Malagasy *Coffea* originate from one or two colonization events. The minimum age of
376 colonization in the former scenario is 5.8 Ma (HPD, 4.1–7.7 Ma; Fig. 2, node 736), whereas
377 two independent arrivals would likely have been more recent. Although the inferred pattern of
378 range expansion in *Bertiera* is not conclusive, it is likely that this genus has been present in
379 the WIOR since 4.4 Ma (HPD, 2.4–6.7 Ma; Fig. 2, node 660). We hypothesize that
380 Madagascar was colonized before the Mascarenes. *Gardenia*, *Hyperacanthus*, *Polysphaeria*,
381 *Tricalysia* and the ancestor of *Galinierea myrtooides* likely colonized Madagascar from Africa

382 within the last 5 Myr. *Catunaregam* is a similarly recent arrival, but according to the Lagrange
383 analysis from Asia. Although Asia is the ancestral area of this genus it should be noted that the
384 phylogenetic relationships between its Asian, African and Malagasy species are not resolved
385 with strong support in this study.

386 The extant Coffeae alliance native to the Seychelles have reached the archipelago
387 either via long-distance dispersal events from the East African mainland (*Rothmannia annae*;
388 and most likely the Aldabran *Polysphaeria multiflora* and *Empogona ovalifolia*, not included
389 in the analysis), or from Madagascar (*Paragenipa lancifolia*, *Paracephaelis trichantha* and
390 *Tarenna sechellensis*). However, it should be noted that *Glionnetia*, one of the outgroup taxa
391 of our analyses (belonging to the Vanguerieae alliance; Ixoroideae; Razafimandimbison *et al.*,
392 2011), represents a clade that is inferred as having dispersed from Asia to the Seychelles (Fig.
393 1). The divergence ages indicate that the colonization events of *Paragenipa* (4.5 Ma, HPD:
394 2.7–6.5), *Rothmannia* (1.7 Ma, HPD: 0.3–3.1), *Tarenna* (1.2 Ma, HPD: 0.4–2.1) and
395 *Paracephaelis* (0.7 Ma, HPD: 0.2–1.3) all occurred within the last 6.5 Ma.

396 The Comoran *Coffea humblotiana*, *Coptosperma nigrescens*, *C. supra-axillare*,
397 *Paracephaelis cinerea*, *Tarenna grevei* and *T. spiranthera* all appear to have dispersed from
398 Madagascar, whereas *Cremaspora triflora*, *Polysphaeria lanceolata* and *P. multiflora* have
399 reached the Comoros from eastern Africa. Unfortunately, no samples of *Coptosperma*
400 *mitochondrioides* or *Polysphaeria lanceolata* from the Comoros were included in our
401 analyses. However, considering the phylogenetic positions of these species (Figs 3 & 5), it
402 can be inferred that the former species dispersed from Madagascar and the latter from Africa.
403 Regarding *Empogona ovalifolia* it is unclear if its distribution in the Comoros and
404 Madagascar is the result of independent dispersal events from East Africa, or stepping-stone
405 dispersal via either the Comoros or Madagascar. The divergence time analysis indicates that
406 all dispersals to the Comoros from Madagascar have occurred within the last 3.4 Ma, and
407 those from Africa within the last 1.6 Ma. *Coptosperma nigrescens* and *C. supra-axillare* have
408 also colonized eastern Africa, possibly by stepping stone dispersal via the Comoros; at least
409 those scenarios are not contradicted by our results. Out-of-Madagascar dispersal to Africa is
410 also found in the *Coptosperma littorale*-*C. rhodesiacum* clade, and probably in *Paracephaelis*
411 (no African material sampled).

412 The Mascarenes are home to *Bertiera*, *Coffea*, *Coptosperma*, *Fernelia* and
413 *Ramosmania*, and the latter two are endemic to this archipelago. *Fernelia* and *Ramosmania*

414 are nested within a clade of East African taxa, and Africa is supported as the ancestral area.
415 The divergence time of the *Fernelia-Ramosmania* clade is estimated to 7.4 Ma (HPD: 4.9–
416 9.7), an age comparable to that of the formation of Mauritius (8 Ma; Duncan, 1990). The
417 crown group, however, is considerably younger (4.1 Ma, HPD: 2.1–6.3 Ma). Notably, the
418 stem age of *Ramosmania* is older than the island of Rodrigues (1.5 Ma; McDougall *et al.*,
419 1965, but see Strijk *et al.*, 2012), to which *Ramosmania* is currently restricted. However, these
420 age estimates are not necessarily conflicting, because we have no estimate of the crown age of
421 *Ramosmania*, and cannot preclude that, until recently, *Ramosmania* was native to Mauritius as
422 well. In contrast, Strijk *et al.* (2012) conjectured an older origin of Rodrigues, and suggested
423 that the island has served as a stepping stone for regional colonization of *Psiadia* (Asteraceae;
424 although their analyses showed a HPD for the crown age of the Rodriguesian taxa that fits
425 well with the young age of the island).

426 *Bertiera* and *Coffea* may have arrived in the Mascarenes in close concert; at least they
427 have a similar estimated crown age of 2.7 Ma (HPD: 1.2–4.2 Ma for *Bertiera* and 1.1–4.5 Ma
428 for *Coffea*). A somewhat younger minimum age estimate of 1.8 Ma (HPD: 0.8–2.8 Ma) for the
429 Mascarene *Coffea* arrival was reported by Nowak *et al.* (2014). Like the *Fernelia-*
430 *Ramosmania* ancestor, *Coffea* is supported as having dispersed from Africa, whereas the
431 ancestral area of *Bertiera* is equivocal, either Africa or Madagascar. *Coptosperma* dispersed
432 out-of-Madagascar, and probably represents the most recent arrival to the Mascarene islands,
433 with a crown age of 0.5 Ma (HPD, 0.1–1.1 Ma). All four groups have dispersed between
434 Mauritius and Réunion, and with the possible exception of the *Bertiera borbonica-B. rufa*
435 clade (crown age: 1.0, HPD, 0.3–1.9), within the last 1 Ma. Compared with the Coffeeae
436 alliance colonization events of the Comoros and Seychelles, the Mascarene radiations appear
437 more diverse in terms of the number of species, although taxonomic inflation may also be part
438 of the explanation. The biogeography of the Coffeeae alliance in the WIOR is discussed in
439 more detail in Appendix S3 in the Supporting Information.

440 In summary, we find that Madagascar has predominantly been colonized by dispersal
441 from (eastern) Africa. Colonizations of the neighbouring archipelagos of the Comoros,
442 Mascarenes and Seychelles, have partly also been from Africa but have mainly been the result
443 of subsequent stepping-stone dispersals from Madagascar, whereas floristic exchange between
444 the WIOR and India or Southeast Asia has not been prominent in the Coffeeae alliance. Most
445 dispersals have occurred in the last 10 Myr, and dispersal opportunities appear to have been

446 sporadic, presumably in part due to a paucity of frugivorous migratory birds. This is also
447 reflected in the high levels of endemism in the region. Following successful colonization,
448 radiation appears to have taken place in some groups and on Madagascar in particular. Most
449 notable both in terms of species number and range of habitats occupied are the radiations of
450 *Coffea* (60 sp.; Davis *et al.*, 2010), *Hyperacanthus* (50 sp.; Rakotonasolo & Davis, 2006) and
451 Pavetteae (70 sp.; De Block, 2003), which are present in most vegetation types of
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453

454

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744

745 **SUPPORTING INFORMATION**

746

747 Additional Supporting Information may be found in the online version of this article:

748

749 **Appendix S1** Supplementary table.

750 **Appendix S2** Supporting data.

751 **Appendix S3** Supplementary discussion.

752

753 **BIOSKETCH**

754

755 This research group focuses on phylogenetic and biodiversity studies of the large tropical
756 plant family Rubiaceae. Author contributions: B.B. conceived the ideas; B.B., K.K. and
757 S.G.R. collected the data; K.K. and N.W. analysed the data, and K.K. led the writing.

758

759 Editor: Peter Linder

760 **Fig. 1.** The Maximum clade credibility (MCC) tree from the BEAST analyses of the combined
761 Coffeae alliance data set. The tree is drawn as a chronogram with node heights representing
762 the mean of the posterior sample of trees. Age estimates are summarized for each node (95%
763 highest posterior density intervals). Well-supported nodes (posterior probability ≥ 0.95) are
764 shown as black bullets. Node numbers correspond to those listed for the biogeographic results
765 (Appendix S2). Relationships within the collapsed clades are detailed in Figs 2–5. Results
766 from the LAGRANGE analyses are shown for nodes of interest. The inferred dispersal events
767 are summarized on the map of the Western Indian Ocean region (WIOR; Lambert projection).
768 The colours of the taxon names represent geographic origin: Africa = black, Americas =
769 orange, Asia-Pacific = magenta, Madagascar = green and Seychelles = cyan.

770

771 **Fig. 2.** Part of the Maximum clade credibility (MCC) tree from the BEAST analyses of the
772 combined Coffeae alliance data set, showing tribes Bertiereae and Coffeae. Age estimates
773 are summarized for each node (95% highest posterior density intervals). Well-supported nodes
774 (posterior probability ≥ 0.95) are shown as black bullets. Results from the LAGRANGE analyses
775 are shown for nodes of interest. The inferred dispersal events are summarized on the map of
776 the Western Indian Ocean region (WIOR; Lambert projection; dashed arrows represent
777 uncertain routes). The colours of the taxon names: Africa = black, Americas = orange, Asia-
778 Pacific = magenta, Comoros = brown, Madagascar = green, Mascarenes = blue and
779 Seychelles = cyan.

780

781 **Fig. 3.** Part of the Maximum clade credibility (MCC) tree from the BEAST analyses of the
782 combined Coffeae alliance data set, showing Octotropideae. Age estimates are summarized
783 for each node (95% highest posterior density intervals). Well-supported nodes (posterior
784 probability ≥ 0.95) are shown as black bullets. Results from the LAGRANGE analyses are
785 shown for nodes of interest. The inferred dispersal events are summarized on the map of the
786 Western Indian Ocean region (WIOR; Lambert projection). The colours of the taxon names
787 represent geographic origin: Africa = black, Asia-Pacific = magenta, Comoros = brown,
788 Madagascar = green, Mascarenes = blue and Seychelles = cyan.

789

790 **Fig. 4.** Part of the Maximum clade credibility (MCC) tree from the BEAST analyses of the
791 combined Coffeae alliance data set, showing tribes Cordiereae and Gardenieae. Age

792 estimates are summarized for each node (95% highest posterior density intervals). Well-
793 supported nodes (posterior probability ≥ 0.95) are shown as black bullets. Results from the
794 LAGRANGE analyses are shown for nodes of interest. The inferred dispersal events are
795 summarized on the map of the Western Indian Ocean region (WIOR; Lambert projection).
796 The colours of the taxon names represent geographic origin: Africa = black, Americas =
797 orange, Asia-Pacific = magenta, Madagascar = green and Seychelles = cyan.

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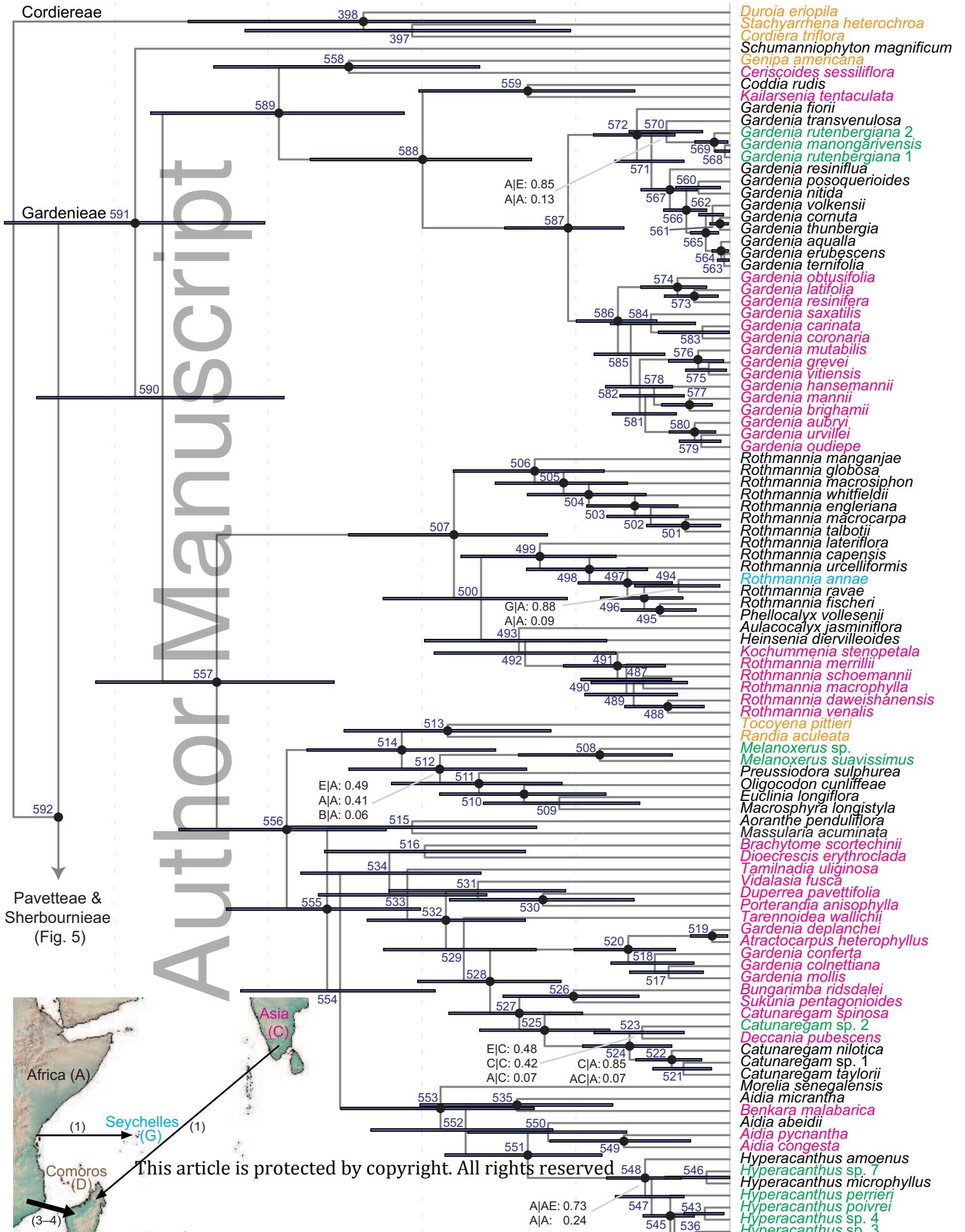
799 **Fig. 5.** Part of the maximum clade credibility (MCC) tree from the BEAST analyses of the
800 combined Coffeae alliance data set, showing tribes Sherbournieae and Pavetteae. Age
801 estimates are summarized for each node (95% highest posterior density intervals). Well-
802 supported nodes (posterior probability ≥ 0.95) are shown as black bullets. Results from the
803 LAGRANGE analyses are shown for nodes of interest. The inferred dispersal events are
804 summarized on the map of the Western Indian Ocean region (WIOR; Lambert projection;
805 dashed arrow represent uncertain route). The colours of the taxon names represent geographic
806 origin: Africa = black, Asia-Pacific = magenta, Comoros = brown, Madagascar = green,
807 Mascarenes = blue and Seychelles = cyan.

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